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PATENT

Attorney Reference Number 4239-60808  
Application Number 09/936,537

## LISTING OF CLAIMS

This listing of claims will replace all prior versions, and listings of claims in the application:

**1. (Previously presented)** A method of selectively inhibiting an immune response to one or more selected antigens comprising:

exposing purified or isolated antigen presenting cells (APCs), which present an antigen against which selective inhibition of an immune response is desired, to an immunosuppressive composition comprising one or more factors secreted by a glioblastoma cell, wherein the one or more factors secreted by the glioblastoma cells induce APCs to induce T cells to undergo apoptosis, and wherein the one or more factors (a) have a minimum molecular mass of about 40 kDa, (b) bind to an anion exchange column, (c) do not bind to a cation exchange column, (d) maintain the ability to induce APCs in the pH range of about 2-11 or following heat exposure up to about 56°C, (e) substantially lose the ability to induce APCs following heat exposure above about 56°C or following trypsin exposure, and (f) are not immunoprecipitated from glioblastoma culture supernatant by neutralizing antibodies against TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, IL-6, calcitonin gene related peptide (CGRP), or M-CSF; and

introducing a therapeutically effective amount of the APCs exposed to the immunosuppressive composition into a subject in whom a selectively inhibited immune response to the antigen is desired, wherein introduction of the APCs inhibits the immune response of the subject to the antigen.

**2. (Currently amended)** ~~The A method of claim 1, selectively inhibiting an immune response to one or more selected antigens comprising:~~

~~exposing purified or isolated antigen presenting cells (APCs), comprising macrophages, monocytes, dendritic cells, and/or B cells, to an immunosuppressive composition comprising one or more factors secreted by one or more glioblastoma cells, wherein the APCs present an antigen against which selective inhibition of an immune response is desired; and wherein incubation of monocytes, dendrites, and B cells the APCs with the one or more factors results in effects comprising:~~

~~—(a) decreased-decreasing expression of MHC class II antigens and CD 80/86 on the surface of the monocytes and the dendritesdendritic cells;~~

~~—(b) increased-increasing expression of IL-10 in the monocytes and dendritesdendritic cells; and,~~

~~—(c) decreased-decreasing the expression of IL-12 in the monocytes and dendritesdendritic cells, and (d) inducing the APCs to induce T cells to undergo apoptosis; -~~

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and wherein the one or more factors (1) have a minimum molecular mass of about 40 kDa, (2) bind to an anion exchange column, (3) do not bind to a cation exchange column, (4) maintain the ability to induce APCs in the pH range of about 2-11 or following heat exposure up to about 56°C, (5) substantially lose the ability to induce APCs following heat exposure above about 56°C or following trypsin exposure, and (6) are not immunoprecipitated from glioblastoma culture supernatant by neutralizing antibodies against TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, IL-6, calcitonin gene related peptide (CGRP), or M-CSF

3. **(Currently amended)** The method of claim 12, wherein the purified or isolated APCs are obtained from a transplant donor, and wherein the APCs express a transplant antigen against which specific inhibition of the immune response is desired.

4. **(Currently amended)** The method of claim 12, wherein the APCs are obtained from a subject, wherein the APCs present an autoantigenic antigen against which specific inhibition of the immune response is desired.

5. **(Original)** The method of claim 4, wherein the purified or isolated APCs are incubated with an autoantigenic peptide, in an amount effective to cause the APCs to present the autoantigenic peptide.

6. **(Cancelled).**

7. **(Currently amended)** The method of claim 12, wherein the APCs are obtained from a donor other than the subject, and the selected antigens are donor-specific antigens present on an allogenic graft.

8. **(Previously presented)** The method of claim 7, wherein the APCs are obtained from a donor of an allogenic graft that is transplanted to the subject, and the introducing step comprises administering the exposed APCs to the subject to specifically inhibit the subject's immune response against an antigen presented by the APCs of the donor.

9. **(Currently amended)** The method of claim 1-2 wherein the antigen presented by the

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APCs is an autoantigenic protein of an autoimmune disease.

10. **(Previously presented)** The method of claim 9, wherein the purified or isolated APCs are obtained from a subject suffering from an autoimmune disease, and the isolated or purified APCs are repetitively exposed to one or more peptide fragments of the autoantigenic protein of the autoimmune disease.

11. **(Original)** The method of claim 10, wherein the autoimmune disease is selected from the group consisting of multiple sclerosis (MS), rheumatoid arthritis (RA), myasthenia gravis (MG), systemic lupus erythematosus (SLE), and insulin dependent diabetes mellitus (IDDM).

12. **(Original)** The method of claim 10, wherein the autoantigenic protein is selected from the group consisting of myelin basic protein (MBP), type II collagen, acetyl choline receptor (AcChoR), nuclear proteins, and pancreatic islet cell antigens.

13. **(Currently amended)** The method of claim 12, wherein the APCs are selected from the group consisting of monocytes, macrophages, and dendritic cells.

14. **(Original)** The method of claim 13, wherein the APCs comprise monocytes.

15. **(Original)** The method of claim 8, wherein the APCs comprise monocytes isolated or purified from the donor's blood.

16. **(Original)** The method of claim 9, wherein the APCs comprise monocytes isolated or purified from the subject's blood.

17. **(Currently amended)** The method of claim 12, wherein the glioblastoma cell is selected from the group consisting of SNB 19 (DSMZ no. 325), A172 (ATCC no. CRL-1620), U87 MG (ATCC no. HTB-14), U138 MG (ATCC no. HTB-16) and U373 MG (ECACC no. 89081403).

18-24. **(Cancelled).**

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25. **(Original)** The method of claim 2, wherein introducing the APCs into the subject comprises administering the APCs by a route selected from the group consisting of intravenous, subcutaneous, intramuscular, and intraperitoneal administration.

26-27. **(Cancelled).**

28. **(Currently amended)** A method of selectively inhibiting an immune response to one or more selected antigens comprising:

exposing purified or isolated antigen presenting cells (APCs), which present an antigen against which selective inhibition of an immune response is desired, to an immunosuppressive composition comprising one or more factors secreted by a glioblastoma cell, wherein the one or more factors secreted by the glioblastoma cell induce APCs to induce T cells to undergo apoptosis, and wherein the one or more factors (a) have a minimum molecular mass of about 40 kDa, (b) bind to an anion exchange column, (c) do not bind to a cation exchange column, (d) maintain the ability to induce APCs in the pH range of about 2-11 and following heat exposure up to about 56°C, (e) substantially lose the ability to induce APCs following heat exposure above about 56°C and following trypsin exposure, and (f) are not immunoprecipitated from glioblastoma culture supernatant by neutralizing antibodies against TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, IL-6, calcitonin gene related peptide (CGRP), or M-CSF; and

introducing a therapeutically effective amount of the APCs exposed to the immunosuppressive composition into a subject in whom a selectively inhibited immune response to the antigen is desired, wherein introduction of the APCs inhibits the immune response of the subject to the antigen;

wherein the glioblastoma cell is selected from the group consisting of SNB 19 (DSMZ no. 325), A172 (ATCC no. CRL-1620), U87 MG (ATCC no. HTB-14), U138 MG (ATCC no. HTB-16), U373 MG (ECACC no. 89081403), T98G (ATCC no. CRL-1690), DBTRG-05MG (ATCC no. CRL-2020), M059K (ATCC no. CRL-2365), M059J (ATCC no. CRL-2366), and U118 MG (ATCC no. HTB-15).

29-33. **(Cancelled).**

34. **(Currently amended)** The method of claim 28, wherein incubation of monocytes, ~~dendrites~~ dendritic cells, and B cells with the one or more factors results in effects comprising:

(a) decreased expression of MHC class II antigens and CD 80/86 on the surface of the

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monocytes and the ~~dendrites~~dendritic cells, without substantial effect on the expression of MHC class II antigens and CD 80/86 on the B cells;

- (b) increased expression of IL-10 in monocytes and ~~dendrites~~dendritic cells; and
- (c) decreased the expression of IL-12 in monocytes and ~~dendrites~~dendritic cells.

35-36. (Cancelled).

37. (Currently amended) The method of claim 42, wherein the glioblastoma cell is selected from the group consisting of T98G (ATCC no. CRL-1690), DBTRG-05MG (ATCC no. CRL-2020), M059K (ATCC no. CRL-2365), M059J (ATCC no. CRL-2366), and U118 MG (ATCC no. HTB-15).